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# Did Dendritic Cell Activation, Induced by Adenovirus-Antibody Complexes, Play a Role in the Death of Jesse Gelsinger?

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We are currently in an era of high hope for the future of human gene therapy. Academic breakthroughs, engagement by a broad range of industries working through first-in-human studies with academics, expansive clinical data, and clearer regulatory paths have fueled the anticipation for success. In general terms, the gene therapy pipeline is directly linked to vector efficacy and the ability to preferentially target a specific cell type or tissue. However, for many diseases, this is less straightforward because intravascular delivery of the gene therapy agent may be required. This comes with the caveat that injection of vectors into the bloodstream exposes the approach to a panacea of possible interactions of the host with the vector that might impact the therapeutic approach. In 1999, a subject with ornithine transcarbamylase deficiency developed lethal systemic inflammation following intra-arterial administration of an adenovirus (Ad) vector. Building on 20 years of improved knowledge of innate immunity to gene therapy vectors, a new study published in this issue of *Molecular Therapy* investigated a potential reason to explain this event.

Ad-based vectors have been extensively used for gene therapeutics and as genetic vaccines via intradermal, intramuscular, or intratumoral injections.<sup>1</sup> By contrast, their utility for intravascular administration has been much more challenging and controversial. Ad vectors are characterized by their relative ease of construction, modification of the capsid, simplicity for clinical grade production, and breadth of cell types that are transduced upon exposure. On the other hand, fundamental questions relating to the effect

on a host either immunologically naive or with anti-Ad immunity are still poorly understood. With respect to vectors derived from human type 5 (HAdV5), a transforming event in the entire field of gene therapy was the death of patient 019, later disclosed as Jesse Gelsinger (see <https://www.sciencehistory.org/distillations/the-death-of-jesse-gelsinger-20-years-later> for a recent article). Mr. Gelsinger was enrolled in a study for gene therapy in the context of ornithine transcarbamylase (OTC) deficiency.<sup>2</sup> Eighteen hours after being infused into the right hepatic artery with  $6 \times 10^{11}$  particles/kg ( $>3 \times 10^{13}$  total particles), his clinical course was marked by systemic inflammatory response syndrome, disseminated intravascular coagulation, and multiple organ system failure, leading to death 98 h post-vector injection. Not only did this event impact the entire field, but it underscored the paucity in our understanding of host-vector interactions, particularly in the context of a host with memory immunity to HAdV5. This fundamental lack of understanding in host-HAdV5 interaction was also compounded by the finding in the STEP vaccine trial using HAdV5 for vaccination against HIV, that subjects with pre-existing antibodies to this virus actually showed increased incidence of infection by HIV.<sup>3</sup>

While the reasons for Jesse Gelsinger's death are likely multiple and complex, 20 years later we have a follow-up study that might shed more light on what happened. In this issue of *Molecular Therapy*, Somanathan et al.<sup>4</sup> revisit the hypothesis that a pre-existing anti-HAdV5 immune response was responsible for a heightened host dendritic cell (DC) activation, which may have been

a driving factor in the inflammatory response. A series of findings in the study are worth noting: the authors first showed that both rabbit polyclonal antibodies to HAdV5 or intravenous immunoglobulin (IV-Ig) (a mixture of sera from human blood donors) enhanced HAdV5-mediated gene transfer into human DC cultures, a finding consistent with several prior reports. Of note, rabbits injected with HAdV5 vectors produced type-specific antibodies directed against the Ad hexon epitopes. This is critical in these assays because at least two other studies showed that anti-hexon antibodies in IV-Ig are responsible for the activation of DCs by HAdV5-IgG complexes. Additionally, the authors showed with the rabbit antiserum that this also led to marked DC activation, measured by CD80 expression and interleukin-6 (IL-6) secretion. Similar findings were observed in mouse bone marrow-derived DCs, although one should caution that HAdV5 can directly activate mouse DCs. Further, when they assessed the sera of three patients that had received HAdV5-based topical gene therapy, they showed that the two subjects that had neutralizing antibodies (NABs) to HAdV5 post-gene therapy also showed enhanced HAdV5-mediated gene transfer in human DCs and concomitant DC activation (similar to observations by others). Moreover, when they assessed 46 sera samples from random human donors, they observed a broad range of NABs and, in some cases, enhanced DC transduction, but DC activation in only 3 sera, a finding seemingly independent of the level of HAdV5 NAB titers. These results need to be interpreted carefully because, if the NAB titer is high in anti-hexon NABs, immunoglobulin-complexed (IC)-HAdV5 will cause the majority of cells to undergo

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pyroptosis (see below). Then, there are few cells remaining to mature and the maturation effect is hidden (for example, see the undiluted serum in Figure 1C of Eichholz et al.<sup>5</sup>). Furthermore, Perreau et al.<sup>6</sup> also showed the importance of FcγR I, -II, and -III in such a process of human DC activation.

To mimic an *in vivo* environment, Somanathan et al.<sup>4</sup> passively infused IV-Ig into mice prior to intravascular HAdV5 administration and demonstrated an increase in circulating pro-inflammatory cytokine IL-6 with kinetics similar to the Gelsinger case. IL-6 levels in these mice injected with HAdV5 alone were, however, 10-fold lower than what others had reported.<sup>7</sup> Finally, they went back to a sample stored from Gelsinger to ask whether the DC hypothesis was possible. Worthy of noting, that this was a frozen whole blood sample taken prior to vector injection, so they used similar whole blood samples to ensure correct controls. Limitations in the quality, quantity, and purity of donor DCs may cause assay variability, but the salient findings are that the blood sample from Gelsinger increased DC transduction and activation, whereas the 6 other samples, in general, all showed an enhanced HAdV5 transduction, but limited enhancement of costimulation and IL-6. The authors therefore proposed that those who harbor an equivalent anti-HAdV5 NAb titer constitute approximately 5%–10% of potential subjects. Alternatively, Jesse Gelsinger may have had a unique distribution of HAdV5 NABs that can target fiber, penton, base, and/or hexon.

As one might expect, conclusions about a substantial event in the field should be treated with caution. As already mentioned, the mechanism by which Ig-complexed HAdV5 induces the maturation and death of human DCs has been described in detail and showed that human DCs undergo activation and pyroptosis via AIM2 (absent in melanoma 2)-dependent activation of an inflammasome.<sup>5</sup> Tran et al.<sup>8</sup> showed that numerous pro-inflammatory cytokines, including IL-6, are released following IC-Ad challenge. Conventional DCs (as modeled by monocyte-derived DCs) make up 0.3% to 1% of leukocytes and therefore are capable of having a significant impact.

Trained immunity, the transient (weeks) metabolic and epigenetic reprogramming of myeloid cells in the bone marrow, may also be a potential avenue to explore.<sup>9</sup> In this scenario, when the monocytes and macrophages are released into the circulation, they are primed for a heightened immune response. While we are unaware of the immune status of Jesse Gelsinger, or OTC patients in general, anyone would have been excited and anxious prior to a life event such as the trial he enrolled in. This could, in theory, have fostered a heightened immune profile and possibly impacted the ensuing cytokine storm. Other potential compounding factors include the ability of HAdV5 to agglutinate human red blood cells. Unlike mice, but similar to rats, human red blood cells have a low level of CAR on their surface.<sup>10,11</sup> It has been known for decades that high doses of HAdV5 can be rapidly lethal in some rat strains, while similar levels do little to mice.<sup>12</sup> This phenomenon, and the broader differences in species dependency in host-HAdV5 interactions, was not fully understood until 10 years after the OTC trial.

As pointed out in the paper, the authors are limited to the single clinical case and single remaining blood sample but do provide more power in some of the *in vitro* experiments and animal studies. Analysis of blood samples from the other subjects could have added power and confidence to the DC transduction and activation hypothesis and link to clinical data (particularly IL-6 levels). Indeed, for a number of reasons, other aspects of the paper were with limited samples and without power and, thereby, no robust statistical assessments. Thus, definitive conclusions must be tempered with utmost caution around robustness and sample sizes. Further, causality in terms of the specific antibody type, the lack of association with NAb titer, and the importance of any findings for other HAdV remain to be understood in detail and require further validation—all important issues for the field.

Certainly, intravascular administration of HAd-based vectors remains a logical approach in many cases for gene therapeutics. Should the findings highlighted herein be validated

further and accepted by the community and regulators, then one might imagine the rationale for screening subjects enrolled on HAdV5-based trials for DC-mediated enhancement in virus transduction and activation. This is likely clearly pertinent for HAdV5-based vectors, but one cannot help to reason that more research is needed to understand the implications for other Ad types, as many are prevalent in the human population through natural infections. Finally, Somanathan et al.<sup>4</sup> speculate that the aforementioned toxicity issues for HAdV5 gene therapy might also be relevant for adeno-associated virus (AAV), where some toxicity has been noted at high vector doses. The situation and kinetics are very different, and one would need to see specific studies on AAV to imply such associations. However, the existence of a link between capsid memory immunity and high-dose intravascular administration for gene therapy is an important one, and one that deserves further attention and rigorous validation. With this new study, a possible link has been suggested for the death of Jesse Gelsinger, an event that is more than simply a lasting memory for the field, but also a case for the importance of immune memory at high dose intravascular HAdV5 gene therapy.

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# CRISPR-Cas9 Disruption of Aquaporin 1: An Alternative to Glaucoma Eye Drop Therapy?

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Glaucoma is a group of optic neuropathies and a leading cause of irreversible blindness worldwide, affecting more than 70 million people, with a rising prevalence in aging populations.<sup>1,2</sup> The hallmark and final common pathway of all forms of glaucoma is progressive retinal ganglion cell (RGC) death and optic nerve degeneration.<sup>3</sup> The pathogenic triggering mechanisms are largely unknown, but key risk factors include intraocular pressure (IOP)-related biomechanical stress.<sup>3</sup> Both medical and surgical therapies are available to control IOP, either by reducing production or increasing drainage of aqueous humor. While these treatments are quite effective, there are shortfalls that may result in persistent progressive vision loss despite therapy.<sup>3,4</sup> In this issue of *Molecular Therapy*, Wu et al.<sup>5</sup> describe a new treatment strategy that could result in effective, long-term con-

trol of IOP. They propose to disrupt aquaporin 1 (AQP1) expression within the ciliary epithelium by adeno-associated virus (AAV)-mediated delivery of a CRISPR-Cas9 system (Figure 1), and they provide proof-of-concept in experimental mouse glaucoma models and cultured human ciliary body.

In healthy eyes, IOP is maintained in a physiologic range by the balance of aqueous humor production by the ciliary body and drainage through the iridocorneal angle (Figure 1). Most patients suffer from hypertensive glaucoma with elevated IOP due to increased aqueous humor outflow resistance.<sup>3</sup> In the most common disease forms, such as primary open-angle glaucoma (POAG), the underlying pathogenesis is still largely unknown and a cure is not available.<sup>4</sup> Current treatments are limited to lowering IOP in order to slow

or prevent further RGC loss and damage to the optic nerve.<sup>3,4</sup> Reduction of IOP is the only proven method to treat glaucoma and slow progression of vision loss; this can be achieved by medical and surgical treatments, including daily eye drops as well as laser and incisional surgeries.<sup>3,4</sup> Low patient adherence rates for long-term self-administration of IOP-lowering eye drops is a major problem that contributes to disease progression despite therapy.<sup>6,7</sup>

Strategies are being developed to address this problem, most importantly by use of sustained drug delivery technologies, with a number of drug implants in pre-clinical and clinical testing.<sup>6</sup> By taking advantage of the latest gene therapy vector and gene editing advances, Wu et al.<sup>5</sup> propose another alternative to eye drops by AAV-mediated intraocular delivery of CRISPR-Cas9 to disrupt the AQP1 gene within the non-pigmented ciliary epithelium, which encodes for a membrane

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